Armed Forces
Radiobiology Research Institute

Health Effects of Embedded Depleted Uranium Fragments

An
Armed Forces Radiobiology
Research Institute
Workshop

15 November 1996

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Edited by
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Contents

Abstract

During Operation Desert Storm (ODS) friendly-fire incidents resulted in patients wounded from embedded fragments of depleted uranium (DU) metal. Existing fragment removal guidelines dictated fragments be left in place unless they were a present or future threat to health. An Armed Forces Radiobiology Research Institute (AFRRI) 1993 review of the potential health effects of allowing DU fragments to remain in place found no compelling evidence to warrant a change in the fragment removal policies. However, sufficient uncertainties existed concerning the health effects of embedded DU fragments to warrant implementation of both patient follow-up and toxicological research programs. The Department of Veterans Affairs (DVA) is conducting a joint DoD/DVA patient monitoring effort; and the DoD is funding a DU research program at AFRRI and at the Inhalation Toxicology Research Institute* (ITRI). A meeting of these groups was held at AFRRI 15 November 1996 to review research efforts to date. This report is a summary of the eight research efforts presented at the workshop.

Toxicological Research Program

AFRRI. The AFRRI research program began in 1993 with a literature review of the potential health effects of allowing DU fragments to remain in wounded individuals. A pilot study was begun in April of 1994 with funding from PM Tank Main Arms Systems, Picatinny Arsenal, to establish the adequacy of a rat model for the study of embedded DU fragments. In December 1994 AFRRI received a 3-year research contract from the U.S. Army Medical Research and Materiel Command (USAMRMC) for partial funding of the AFRRI research effort on DU toxicity. At the time of this workshop AFRRI was in the second year of the 3-year study. The findings presented here represent results obtained from rats implanted with DU pellets for 6 months. In the next year data from the groups evaluated at 12 and 18 months will be collected and analyzed.

Chronic exposure to DU as a result of release from implanted pellets in rats was not as nephrotoxic as originally anticipated. The concentration of DU in the kidneys of male rats was well above the level that is known to be nephrotoxic in rats for acute intakes of uranium. There was, however, no physiologic or histologic indication of kidney damage at the 6-month time point, although later expression of damage cannot be precluded.

DU crossed the blood-brain barrier in rats. DU concentrations in the brain rose in a dose- and time-dependent fashion. Physiological changes of DU-implanted rats occurred in the hippocampus, a region of the brain associated with learning and memory. The observed changes in neuronal function at the 6-month time point raise concerns that, like lead, DU may cause cognitive deficits. This has implications not only for the exposed adult but also for the developing fetus.

Because of concern about the effect of DU on the developing fetus, a pilot study using DU-implanted pregnant rats was initiated in August 1994 with funding from the U.S. Army Environmental Policy Institute. Preliminary results indicate that DU accumulates in the placental barrier and crosses to the fetus. In October 1996, AFRRI received a contract from the USAMRMC, Women's Health Issues Program, for a full study of this problem.

*In 1997 renamed the Lovelace Respiratory Research Institute

AFRRI has also completed the first phase of an *in vitro/in vivo* carcinogenesis study. A human osteoblast cell line exposed to DU *in vitro* resulted in transformation of the cells. Furthermore, these cells produced tumors when injected into immunologically deficient mice. A number of oncogenes were also expressed in a time- and dose-dependent fashion after DU exposure. The level of oncogenesis and mutagenesis was significantly higher than that expected from radiation alone and is indicative of the potential for chemical carcinogenesis similar to that found with other heavy metals. The carcinogenic potential of DU is higher than that of lead and nickel.

ITRI. A second research program to assess the possible carcinogenic effect of DU in animals is currently being performed at the Inhalation Toxicology Research Institute (ITRI), which is part of the Lovelace Laboratories. This laboratory has completed 2 years of a 3-year study that also began in fiscal year 1995 with USAMRMC funding.

This project is designed to assess the risk of carcinogenesis related to the radiation component of depleted uranium in a rodent model. The program was designed initially to test carcinogenesis by implanting DU metal plates subcutaneously. The results indicate that the subcutaneous implant model is not appropriate to study the carcinogenesis of implanted DU fragments in rats owing to the solubility and dispersion of the metal. An alternate method, intramuscular implantation of DU fragments, will be used to study the carcinogenic effects of implanted DU fragments.

DVA Patient Monitoring Program

Baltimore VA Medical Center. The ODS patient monitoring program began in 1992 with a medical surveillance follow-up study of 33 injured Persian Gulf War veterans. ODS veterans with embedded DU are still excreting DU in their urine at a constant rate almost 6 years after being injured. The urinary uranium levels are below the threshold for acute toxicity. To date, no significant adverse health effects attributable to DU have been noted. A VA sponsored research program for detection and measurement of DU *in situ* using x-ray fluorescence and whole-body counting is being developed at McMaster University in Canada.

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Introduction to the Problem

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The technological superiority of American weapons systems during the Persian Gulf War was demonstrated by the rapid and decisive defeat of the enemy forces. That conflict saw the first documented combat use of depleted uranium (DU) munitions. Friendly-fire incidents involving these weapons caused a number of U.S. injuries, some of which were from DU fragments. There are significant radiological and toxicological unknowns associated with leaving these fragments in place for the expected remaining lifetime (40–50 years) of these soldiers [1].

Depleted uranium is a radioactive, pyrophoric, heavy metal that is about 1.7 times the density of lead (19 g/cm^3 versus 11.35 g/cm^3). DU is obtained as a byproduct of the enrichment process for weapon- and reactor-grade uranium (^{235}U). ^{235}U and ^{234}U are reduced from 0.72% and 0.006%, respectively, in natural uranium to 0.2% and 0.001%, respectively, in DU. The remainder is ^{238}U (approximately 99.8%). DU emits alpha (α), beta (β), and weak gamma (γ) radiations. DU presents minimal external hazard because its radioactivity is very low and the fraction of penetrating radiation emitted per decay is small (<1%) [2–9]. The α radiations present no external hazard because the dead layers of skin stop them. β radiations present a hazard only if the munitions are in contact with the skin. On the other hand, internalized DU presents potential radiological and chemical hazards. The α and β radiations are the primary contributors to the internal radiological hazards. Because of its low radioactivity, DU is one of the few radioactive materials whose occupational exposure is based on its chemical, not radiological, toxicity [4].

One of the primary military uses of DU is in kinetic energy (KE) penetrators to defeat armored vehicles [5,10]. There have been six previous assessments [2,5–9] of the health and environmental risks of DU penetrators. However, while recognizing the possibility of internal injuries from DU, these studies did not directly address this potential problem. Such injuries may occur when injured individuals internalize DU via inhalation, ingestion, wound contamination, or embedded fragments. Inhalation and wound contamination occur because the penetration process results in small airborne particulates that are inhaled. They also produce surface contamination of personnel. Embedded fragments may occur when the penetrator begins to disintegrate after impact with armor, forming high-velocity shards of DU.

To date, hazard assessments have focused on the risks from inhalation [2,5,11–18]. The metabolic models required to estimate the chemical and radiological risks from these exposures are well developed and are based on a large body of animal experimentation and human epidemiological studies [2,5–9,19–22].

In contrast, the long-term health risks of allowing DU fragments to remain embedded in the injured soldier have not been studied. The potential medical significance of embedded DU shrapnel was recognized early in the development of these munitions [8], but the need for research to define these risks was discounted because of the almost 100% fatality rate assumed for personnel inside vehicles penetrated by DU munitions [2,5,8]. However, one of the lessons learned from the

Persian-Gulf War was that personnel may survive penetrations of armored vehicles and many may have wounds with embedded DU fragments [5,23].

The unfortunate friendly-fire incidents involved 15 Bradley Fighting Vehicles (BFV) and 14 Abrams tanks struck by U.S.-fired DU penetrators [23]. Some of the soldiers injured in these incidents were reported to have wounds with embedded DU fragments [23]. Using standard surgical guidelines, many of these fragments were not removed because the risks of surgery were considered too great [23,1]. These guidelines were established based on experience with standard (non-uranium, non-radioactive) fragmentation injuries.

A 1993 review of the potential hazards of embedded DU [1] concluded that there were sufficient uncertainties regarding the long-term chemical and radiological effects to warrant the medical follow-up of current patients and the initiation of research to define the consequences of allowing the fragments to remain in place. The Office of the Army Surgeon General (OTSG) and the Department of Veterans Affairs (DVA), in conjunction with AFRRI, have implemented the medical follow-up of current patients with DU fragments [24].

The results of the patient monitoring protocol to date highlight the potential for long-term radiological and toxicological health effects caused by embedded DU. An analysis of bioassay data from injured soldiers revealed urine uranium concentrations as high as 30 μg per liter of urine 2 years after they were injured [25].

There is further need for this research effort because the potential exists for significantly higher levels of internalized uranium in patients from future battles. These injuries may well involve female as well as male troops, and present an acute treatment problem to the military as well as a long-term problem for the DVA.

References

1. Daxon E, Musk J (1993) Assessment of the risks from imbedded depleted uranium fragments. Armed Forces Radiobiology Research Institute Technical Report, AFRRI TR 93-1

2. AMCCOM TASK GROUP (1990) Kinetic energy penetrator long term strategy study (Abridged), 24 July

3. Military Specification (1991) MIL-U-70457, Notice 1, 28 January

4. Code of Federal Regulations (CFR) Title 10, Part 20

5. Shelton SP, Daxon EG, *et al.* (1994) Summary Report to Congress: Health and environmental consequences of depleted uranium use by the U.S. Army. The Army Environmental Policy Institute, Atlanta, June

6. National Materials Advisory Board, National Research Council (1971) Report of the ad hoc panel on depleted uranium of the Committee on the Technical Aspects of Critical and Strategic Materials. National Academy of Sciences, Washington, D.C., Publication NMAB-275

7. National Materials Advisory Board, National Research Council (1979) Comparison of DU and tungsten for use as kinetic energy penetrators. National Academy of Sciences, Washington, D.C., Publication NMAB-350

8. Special Report: Medical and environmental evaluation of depleted uranium (1974) Joint Technical Coordinating Group for Munitions Effectiveness, Volume I

9. Report of Committee (1979) A hazard evaluation of the use of depleted uranium penetrators. The Pierre Committee Report, April

10. Pengelley R (1994) Tank ammunition development. International Defense Review, 27:39-46

11. Filszar RL, Wilsey EF, Bloore EW (1989) Radiological contamination from impacted Abrams heavy armor. Ballistics Research Laboratory, Aberdeen, MD, Report BRL-TR-3068

12. Wilsey EF, Bloore EW (1989) M774 Cartridges impacting armor-bustle targets: depleted uranium airborne and fallout material, Ballistics Research Laboratory, Aberdeen, MD, Report BRL-mR-3760

13. Bloore EE, Wilsey E (1979) Tank burn test, operation hot box. Ballistic Research Laboratory, Aberdeen Proving Ground, MD, DU-1

14. Chambers D, Markland D, Clary M, Bowman R (1982) Aerosolization characteristics of hard impact testing of depleted uranium penetrators. Ballistic Research Laboratory, Aberdeen Proving Ground, MD, ARBRL- TR-02435

15. Glissmeyer J, Hooker JC (1979) Characterization of airborne uranium from test firings of XM774 ammunition. Richland, WA, Pacific Northwest Laboratory, PNL-2944, UC-35

16. Jette S, Mishima J, Hadlock D (1990) Aerosolization of the M839A1 and XM900E1 rounds fired against hard targets. Richland, WA, Pacific Northwest Laboratory, PNL-7452

17. Ensminger D, Bucci S (1980) Procedures to calculate radiological and toxicological exposures from airborne releases of depleted uranium. The Analytic Sciences Corporation, Reding MA, TR-3135-1

18. Mishima J, *et al.* (1985) Potential behavior of depleted uranium penetrators under shipping and bulk storage conditions. Richland, WA, Pacific Northwest Laboratory, PNL-5415, UC-41

19. Limits for intakes of radionuclides by workers (1981) Pergamon Press, N.Y., ICRP Publication 30

20. Recommendations of the International Commission on Radiological Protection (1977) N.Y., Pergamon Press, ICRP Publication 26(1) No. 3

21. National Research Council, Committee on the Biological Effects of Ionizing Radiation (1990) Health effects of exposure to low levels of ionizing radiation, BEIR V. National Academy Press, Washington, D.C.

22. National Research Council, Committee on the Biological Effects of Ionizing Radiation (1989) Health risks of radon and other internally deposited alpha-emitters, BEIR IV. National Academy Press, Washington, D.C.

23. Operation Desert Storm: Army not adequately prepared to deal with depleted uranium contamination (1993) GAO Report, GAO/NISIAD-93-90

24. Daxon E (1993) Protocol for monitoring Gulf War veterans with imbedded depleted uranium fragment. Armed Forces Radiobiology Research Institute, Technical Report, AFRRI TR 93-2

25. Results of analyzing urine bioassay specimens for uranium (1994) Memorandum for Office of the Surgeon General, PSP (Interim Report) from the U.S. Army Environmental Hygiene Agency, APG, MD, 20 April

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Depleted Uranium Distribution and Toxicology in a Rodent Model

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Previous studies have examined the toxicity associated with uranium exposure through inhalation, ingestion, and injection. Until the Gulf War resulted in injuries from depleted uranium (DU) fragments, toxicity associated with embedded DU had not been considered. We are evaluating kidney, behavioral, and neural toxicity associated with implanted DU pellets. In addition, we are assessing tissues for histological changes and for uranium content using a rodent model.

Male Sprague-Dawley rats were assigned to five experimental groups: (1) nonsurgical controls, (2) control (20 1-mm x 2-mm chemically inert tantalum (Ta) pellets), (3) low-dose DU (four 1-mm x 2-mm DU and 16 Ta pellets), (4) medium-dose DU (10 DU and 10 Ta pellets), and (5) high-dose DU (20 DU pellets). Pellets were surgically implanted into the gastrocnemius muscle of both hindlimbs. Dr. A. Fucciarelli measured uranium in tissue by kinetic phosphorescence analysis at Pacific Northwest Laboratories.

To date, data have been analyzed at 30 days and at 6 months after implantation. Examination of the pellets *in situ* revealed fibrous tissue adhering to the DU but not to the Ta pellets. Uranium levels (figure 1 and figure 2) were high and dose-dependent in kidney, urine, and bone. Unexpectedly, uranium was found in the brain of DU-implanted animals

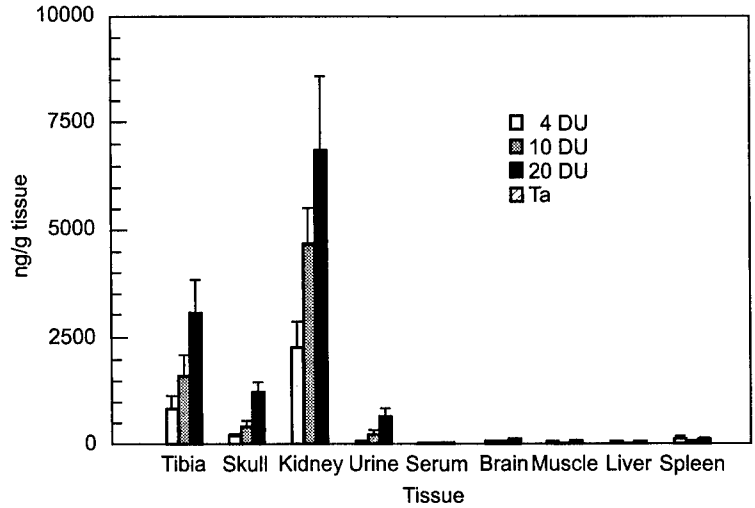

Figure 1. Uranium distribution in rat tissue 6 months after DU pellet implantation. (Bars representing data from Ta-implanted animals are too small to be seen on this graph).

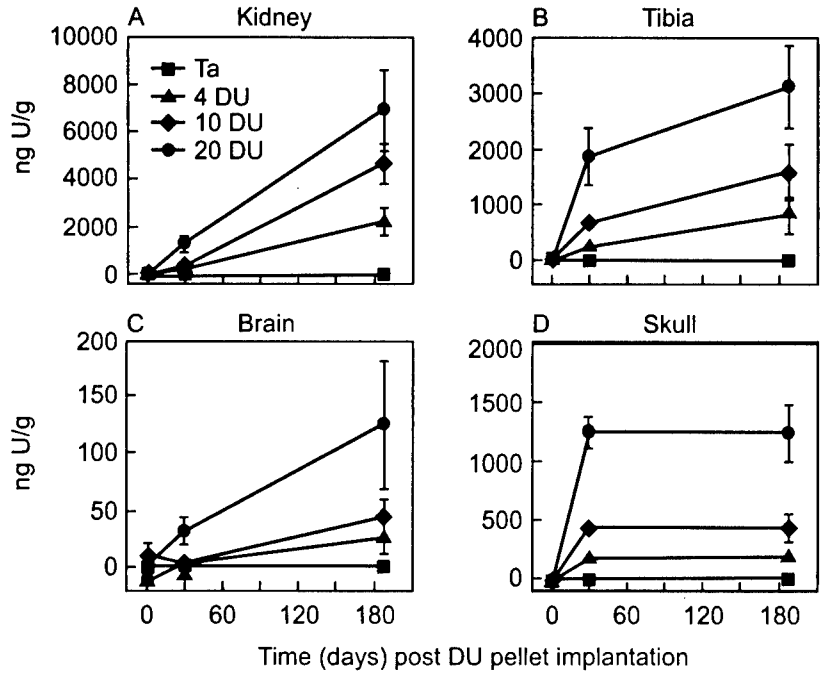

Figure 2. Changes in uranium levels in the kidney, tibia, brain, and skull for 6 months post DU-pellet implantation.

(figure 2c). At 6 months, uranium levels in the kidneys were 0.003 ± 0.001 μg/g in controls, 2.3 ± 0.6 μg/g in low-dose animals, 4.7 ± 0.8 μg/g in medium-dose animals, and 6.9 ± 1.7 μg/g in high-dose animals. The urine levels were 2.0 ± 0.7 μg/l in controls, 46 ± 13 μg/l in low-dose animals, 243 ± 52 μg/l in medium-dose animals, and 674 ± 156 μg/l in high-dose animals. Kidney levels in the high- and medium-dose animals exceeded the 3-μg/g level set by the NRC for renal damage. These urine uranium levels are in the range of clinical and toxicological interest since the highest concentrations measured in the urine of Gulf War veterans were approximately 30 μg/l. Despite these high levels, no evidence of kidney toxicity was noted. Uranium miners have been reported with urine levels near 200 μg/l. The chronic exposure associated with implanted DU pellets may be better tolerated than acute exposures to uranium which demonstrated toxicity at kidney levels as low as 0.7μg/g.

Behavioral and neural toxicity were evaluated through a functional observational battery, locomotor activity, grip strength, passive-avoidance learning, peripheral nerve conduction velocity, and hippocampal field potential recordings. Between 23–26 weeks, body weight in high DU-dose animals was significantly lower than controls (figure 3). No behavioral changes were

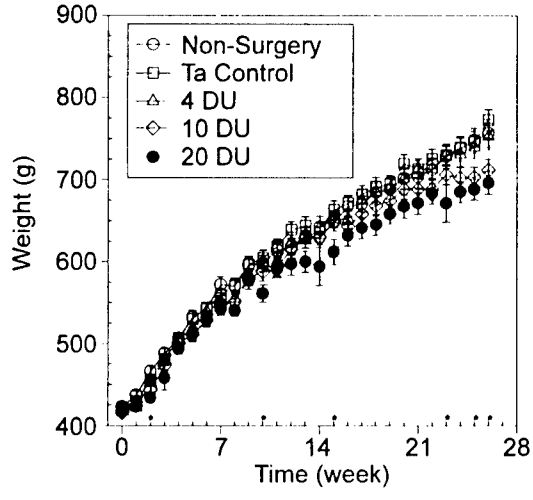

Figure 3. Body weight measured over 28 weeks after DU-pellet implantation. Note that body weight in high-dose animals was significantly lower than in controls between 23 and 26 weeks after DU-pellet implantation.

evident. However, excitability of hippocampal neurons, as determined by electrophysiological analysis, was reduced in the high DU dose animals at 6 months (figure 4). The hippocampus is an area of the brain associated with memory and learning.

These data suggest that renal toxicity may be less of a hazard than originally anticipated. However, cognitive deficits need to be considered. The 12- and 18-month time points will be examined in future experiments.

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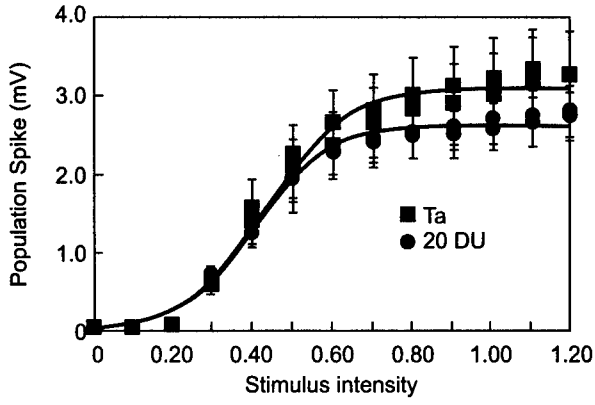

Figure 4. Decrease in population spike in hippocampus of rats 6 months after implantation with 20 DU pellets. Electrophysical properties of neurons are altered.

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Depleted Uranium Health Effects: Transformation, Mutagenicity, and Carcinogenicity

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Limited data exist to permit an accurate assessment of risks for carcinogenesis and mutagenesis from depleted uranium (DU) embedded fragments or inhaled particulates. The Armed Forces Radiobiology Research Institute (AFRRI) has ongoing Army-funded studies that are designed to provide information about DU's potential toxicity. The Army is also funding and conducting a long-term health-effects project with ITRI that should contribute to the understanding of the carcinogenic potential of DU. However, data obtained from AFRRI DU transformation and mutagenicity studies suggest the need for further work since a number of questions are not addressed in the ITRI project. Recent results from DU animal studies at AFRRI suggest there are potential biomarkers for carcinogenesis. These biomarkers have possible applicability for assessing the carcinogenic risk from embedded DU fragments, a current military medical goal. If the studies underway demonstrate that DU is a carcinogen, the development and identification of these biomarkers should be pursued.

Quantitative and qualitative *in vitro* transformation studies are widely used to assess the carcinogenic potential of radiation and chemical hazards. Using a human cell-model system, AFRRI has demonstrated that DU-uranyl chloride can transform cells to the tumorigenic phenotype (table 1).

Morphological, biochemical, and oncogenic changes that are consistent with tumor-cell behavior characterize this transformation. In addition, using the bacterial strain reversion and the unscheduled

Table 1. Transformation of human cells to the tumorigenic phenotype: Comparison of depleted uranium to other heavy metals.

	Transformation rate			Tumorigenicity	
Metal	Cnnc metal (μM)	Survival fraction	Transformation frequency per survivor (x 10^9)	Type of cells injected	Number of tumors formed per number animals injected
None	0	1.0	4.2	HOS control	0/12
DU-uranyl chloride	10	0.95	40.2	HOS w/*met* oncogene	0/12
Nickel sulfate	20	0.94	29.9	HOSw/*ras* oncogene	4/12
Lead acetate	20	0.95	21.0	HOS transformed by lead	1/10
				HOS transformed by nickel	4/12
				HOS transformed by DU	8/19

DNA synthesis (UDS) assays, two tests recommended by the EPA to assess mutagenicity, DU-uranyl chloride was shown to be a mutagen. DU-uranyl chloride was genotoxic to human cells since the frequencies of both sister chromatid exchange (SCE) and micronuclei were higher in DU-treated cells.

Several important unanswered questions can be addressed with additional cellular experiments. Military risk standards for DU exposure are based on the radiological component of DU. However, when DU is internalized in human tissues via inhalation, ingestion, or wounding (e.g., DU shrapnel), the chemical component of DU may be as important as the radiological component. It first needs to be determined if the DU-induced effects on cells (e.g., transformation to the tumorigenic phenotype) are different from that expected from alpha particle radiation exposure alone. A battery of cellular experiments similar to those currently underway could help to partially answer this question. In conjunction with these studies, the *Humm Code* is being used to determine the radiation dose. Secondly, previous *in vitro* and *in vivo* studies with other metals have demonstrated that both solubility and the valence state of the metal are critical to its mutagenic and carcinogenic potential. The importance of solubility and valence state in DU-induced cell transformation is unknown. Additional *in vitro* studies are needed to provide this information for DU compounds. An investigation of the ability of DU to participate in the generation of reactive oxygen species, such as $^{\bullet}OH$, and H_2O_2, is essential to assess DU's carcinogenic potential. Reactive oxygen species and the resultant oxidative DNA damage have been shown to be crucial to carcinogenesis. Furthermore, most mutagens and carcinogens are thought to induce genetic changes by interacting with DNA and causing some critical lesion formation; however, there is little information regarding DU exposure and DNA damage. A determination of critical lesions such as DNA-protein cross links and DNA adducts would contribute to the understanding of the carcinogenic and mutagenic potential of DU.

Studies with DU-embedded animals demonstrated that increased tissue uranium content was associated with aberrant activation of several of the oncogenes and tumor suppressor genes associated with preneoplastic lesions and human carcinogenesis (figure 1A and B). Specific point mutations in these genes have also been identified. In contrast, tissues from animals with tantalum implants did not show this aberrant oncogene pattern. Oncoproteins and tumor suppressor proteins were also found in the serum of animals with DU pellets. These proteins were not detected in serum from animals implanted with tantalum. Mutagenicity tests with animal urine indicated that urine with a high uranium content was mutagenic. In addition, cytogenetic analysis of lymphocytes showed that embedded DU could be correlated with genotoxic damage. However, the assessment of carcinogenic potential cannot be fully answered with one animal model.

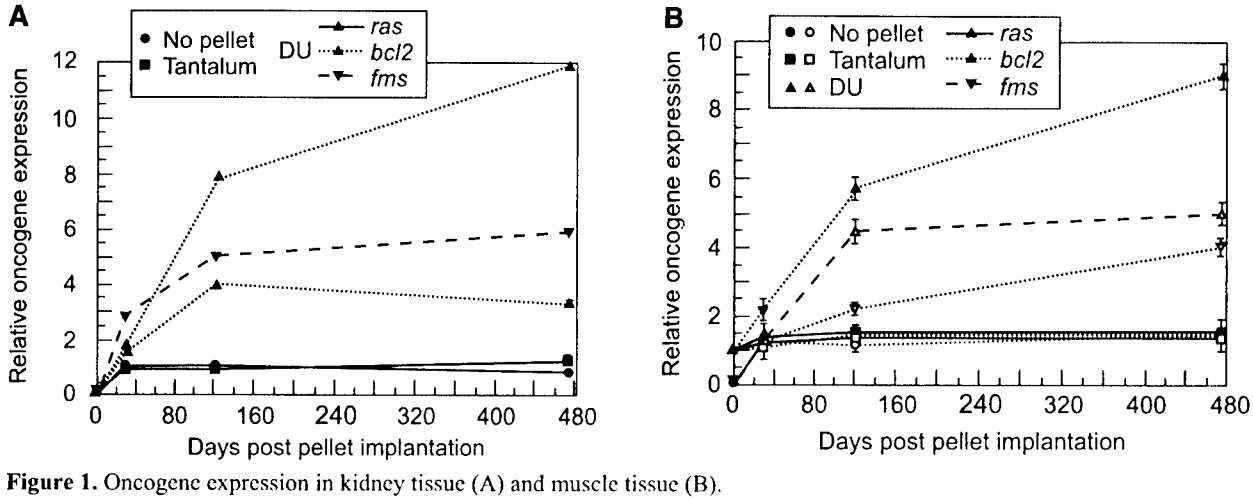

Figure 1. Oncogene expression in kidney tissue (A) and muscle tissue (B).

As shown by earlier carcinogenesis studies involving metal, chemicals, and radiation, what is harmful to one animal model may not have an effect on another. Additionally, differences in spontaneous tumor formation need to be considered when choosing an animal model for carcinogenesis studies. Certainly a study should be considered where the molecular events, such as oncogenesis, mutagenesis, and genotoxicity, crucial to the development of neoplastic disease, are measured in conjunction with animal carcinogenesis. Not only can the carcinogenic potential of DU be assessed in such an investigation, but information can be obtained that may lead to the development of biomarkers for neoplastic tissues.

Cancer development is a multistep process. Specific molecular events at the stages preceding tumor development can lead to detection of highly prevalent biomarkers. Recent advances have been made in detecting these molecular changes in easily accessible body fluids such as urine, sputum, serum, and saliva. This type of non-invasive sampling allows for the large-scale screening of patients who may be at increased risk of developing cancer. Serum and urine samples obtained from soldiers with DU shrapnel could be analyzed for the presence of biomarkers that are consistent with preneoplastic characterization. Combined analysis of multiple markers has the potential to assist in identifying changes in normal tissues during the stages that precede tumor development. Identification of multiple biomarkers appears warranted since it is likely to provide better assurance of detecting changes that are associated with malignancy rather than with mutation events—thereby enhancing their clinical value to risk assessment.

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Depleted Uranium-Induced Immunotoxicity

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A wide variety of heavy-metal ions have been shown to be immunotoxic. For example, mercuric ions induce a significant loss in viability of monocytes, initiating cytotoxic changes associated with programmed cell death. Mercury has also been shown to reduce T-cell proliferation, as well as inhibit the expression of immunoglobulin receptors on B-cells. Cadmium, chromium, lead, and gadolinium have been shown to decrease macrophage viability as well as phagocytotic ability. Additionally, prolonged exposure to nickel, chromium, and cobalt can induce hypersensitivity. Despite the large volume of work on the immunotoxic effects of heavy metals, there apparently have been no previous studies to determine the immunotoxic effect of uranium.

There are many possible routes for internalizing depleted uranium on the battlefield. Of primary concern are embedded fragments of depleted uranium from shrapnel wounds. Obviously, immune-system cells are going to be intimately involved in the process of wound healing. Shortly after an injury, circulating polymorphonuclear (PMN) leukocytes start to appear in the wound. PMNs are the first blood leukocytes to arrive. Their numbers peak in approximately 24 to 48 hours, followed by a rapid decrease unless infection is present. Their primary function is to phagocytize bacteria and other foreign material in the wound. However, their presence is not essential for normal wound healing. The macrophages are the next cellular elements to arrive at the wound. They appear approximately 48 hours after the injury. Their numbers peak 3 days later. Unlike PMNs, macrophages remain in the wound until healing is complete. T-cells, the third immune cell to enter the wound, do so some time later (around 5 days after the injury). In contrast to the PMNs, the presence of both macrophages and lymphocytes in the wound is critical for the process of normal healing. Because of the mechanisms involved in wound healing, immune system cells are going to be in extensive contact with embedded depleted uranium fragments.

Experimental Approach

Our experimental approach is to utilize primary cell cultures of rodent thymocytes, splenocytes, and macrophages and the established cell lines MOLT-4 (human T-cell leukemia), Raji (human B-cell lymphoma), and J774.1 (mouse macrophage). Cells were treated with DU-uranyl chloride or nitrate for various times at concentrations up to 100 μM. The trypan blue dye exclusion method or the MTT assay determined viability of both the treated and untreated cells. Determination of the mode of cell death was accomplished by morphologic or flow cytometric examination, DNA agarose gel electrophoresis, and biochemical quantitation of fragmented DNA using the DNA-specific dye Hoechst 33258 and a sensitive fluorometric assay developed in this laboratory. Functional tests of immune-system cells will be conducted using the National Toxicological Program/Environmental Protection Agency guidelines for determining immunotoxicity. The presence of uranium in cells was determined histochemically using 2-(5-bromo-2-pyridylazo)-5-diethylamino phenol (PADAP) and a procedure developed in this laboratory.

Results

Treatment of mouse thymocytes and splenocytes, as well as MOLT-4 and Raji cells, with DU-uranyl chloride had no significant effect on viability. In addition, there were no signs that apoptosis was occurring in the treated cells. However, treatment of J774.1 cells (mouse macrophage cell line) with various concentrations of DU-uranyl chloride for up to 72 h resulted in a dose-dependent decrease in viability. Morphologically, the treated cells exhibited the characteristics associated with programmed cell death or apoptosis (i.e., cytoplasmic shrinkage, nuclear disruption, plasma membrane perturbations, and production of apoptotic bodies). Biochemical quantitation of DNA isolated from the treated macrophages exhibited a higher percentage of fragmented DNA than did DNA from untreated cells. In addition, uranium treatment interfered with the ability of the macrophages to phagocytize bacteria; although it did not interfere with the ability of the cell to kill the bacteria once internalized. Peritoneal macrophages isolated from rats implanted with DU or tantalum pellets showed no difference in their phagocytic abilities. Finally, a histochemical staining procedure has been developed that allows the cellular location of internalized uranium to be determined. Uranyl chloride-treated J774.1 cells showed a distinct cytoplasmic staining pattern. Untreated cells did not stain.

Conclusions

Thus far the results of this pilot study have shown that uranyl-chloride treatment had no effect on thymocyte or splenocyte viability. However *in vitro* DU-uranyl chloride-treated macrophages showed a dose-dependent decrease in viability and exhibited characteristics of programmed cell death. The treated macrophages also showed a dose-dependent decrease in their ability to phagocytize bacteria; however, antimicrobial or cell kill ability was not impaired. PADAP staining of the uranium-treated macrophages showed an intense cytoplasmic staining while untreated cells did not stain.

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Fetal Development Effects

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In utero exposure to uranium has recently been shown to produce both fetal and developmental toxicity. For example, administration (s.c.) of uranium in the form of uranyl acetate dihydrate (0.5–2.0 mg/kg/d) to gravid (pregnant) mice from gestational days (GD) 6–15 leads to significant decreases in both maternal weight gain and fetal body weights at GD 18 [1]. Soft tissue and skeletal examination of the fetuses also revealed a significant increase in the occurrence of renal hypoplasia in all uranium-treated groups. Skeletal anomalies in these mice included bipartite sternebrae, dorsal hyperkyphosis, and incomplete ossification of several bones. Similar skeletal malformations were also seen following daily oral administration of uranyl acetate dihydrate (5–50 mg/kg/d) in gravid mice during the same period of gestation [2].

While the above results examined the effects of uranium on prenatal development, several studies have been conducted to evaluate the effects of uranium on postnatal development (from birth to age 21 days) [3,4]. Significant decreases in body weight and body length in the offspring of mice treated with 25 mg/kg/d for 14 days prior to mating have been reported [4]. There were also significantly more dead young per litter at this uranium dose at both birth and day 4. Uranyl acetate given orally to gravid mice from GD 13 to 21 days following parturition significantly increased liver weights in all offspring of the uranium-treated groups (5.0–50.0 mg/kg/d) and decreased mean litter size on day 21 in the highest dose group (50 mg/kg/d). However, developmental parameters such as pinna detachment, incisor eruption, and eye opening were unaffected [3].

Unfortunately, uranium levels in dam, fetus, or placenta were not measured in any of these fetal and developmental toxicity studies. To determine the effects of embedded DU on a developing fetus, it is important to know the *in utero* uranium exposure level. However, little work has been done to examine the cross-placental transfer of uranium [5,6]. While there are distinct anatomical differences between the rodent placenta and the human placenta, little correlation has been shown between the anatomic classification of the placenta and the transfer of xenobiotics between mother and fetus [7]. In rodents and primates the placenta may act as a barrier that limits or prevents many toxicological insults to the fetus. This does not appear to be the case with uranium. When ^{233}U was administered intravenously to pregnant rats, almost identical levels of uranium were found in the placenta and fetus [8], indicating little discrimination for uranium by the placenta. The soft-tissue levels of uranium in 19- to 20-day-old fetuses were equal to or greater than the maternal liver concentrations. Immature bone also exhibited a greater deposition of uranium than did the adult bone [6].

While previous research has demonstrated that the placenta does not act as a barrier to prevent the transfer of uranium from the mother to the fetus [8], the degree of fetal exposure from maternal implanted DU is unknown. The current study was designed to address this question by determining the uranium levels in the placenta and the fetus. This study also determined if the DU pellets impact the dam's ability to become pregnant and carry her litter to term.

Materials and Methods

Fifty-four female Sprague-Dawley rats (Charles River) weighing 250–300g were surgically implanted with one of four doses: 12 tantalum pellets,

Table 1. Effects of depleted uranium on maternal parameters.

Variable	No Surgery	0 DU	4 DU	8 DU	12 DU
# Dams bred	16	16	13	17	14
Days to pregnancy (SEM)	3.9 (± 1.76)	2.08 (± 0.23)	3.36 (± 1.11)	4.36 (± 1.42)	4.9 (± 1.97)
Mean weight gain (g)	133.79 (± 8.13)	138.32 (± 6.49)	143.26 (± 4.69)	138.75 (± 4.38)	145.22 (± 6.89)
Mean food intake (g)	23.44 (± 0.67)	24.51 (± 0.68)	24.27 (± 0.52)	23.67 (± 0.79)	24.71 (± 0.71)
Mean water intake (ml)	43.85 (± 2.59)	44.45 (± 2.60)	46.33 (± 2.10)	48.10 (± 1.91)	44.84 (± 1.50)

4 DU and 8 tantalum pellets, 8 DU and 4 tantalum pellets, and 12 DU pellets. There was also a non-surgery control group. At all times any rat receiving pellets had a total of 12 pellets implanted in order to keep the number of the implantations approximately equal in all surgery rats.

Experimental females were housed with non-treated male rats in cages with two females for each male. Gestational Day (GD) 0 was determined by the presence of sperm in the vaginal washing. At this time the females were removed from the cages and housed individually. From GD 0 until GD 20, pregnant rats were monitored daily for weight gain, food intake, and water intake. These parameters were used as measures of maternal toxicity of the DU pellets. On GD 20, the dams were euthanized. Dams were immediately cesarean-sectioned, and the uterine horns were removed. Fetuses were dissected out, and all the placentae for that litter collected. The uterine horns were examined for any resorption sites. Litters were examined; and a record was made of (1) total number of fetuses, (2) the number of viable fetuses, (3) sex ratio, and (4) any overt signs of teratological effects. All offspring of the litter were analyzed for uranium levels. The placentae from all pups were collected and pooled for uranium analysis for each litter. One male and one female pup were separated out and used for analysis of whole fetus. The rest of the litter was used for determining uranium tissue levels. Quickly the liver and kidneys were dissected out of these pups. These tissues were pooled for the entire litter, homogenized, and sent to Quanterra, Inc., Richland, WA, for further analysis of uranium content.

Results

Tables 1 and 2 present the data on the effects of the DU levels on maternal and litter parameters. From these data, there appear to be no effect of

Table 2. Effects of depleted uranium on litter data.

Variable	No surgery	0 DU	4 DU	8 DU	12 DU
Total # fetuses	13.8 (± .79)	13.5 (± .78)	14.8 (± .54)	15.5 (± .53)	15.0 (± 1.09)
# Males	6.6	6.3	8.5	8.7	7.5
# Females	7.2	7.2	6.3	6.8	6.5
# Non-viable	0	1	1	2	0
Average pup weight	3.596 (± .20)	3.164 (± .09)	3.658 (± .27)	3.390 (± .09)	3.378 (± .08)

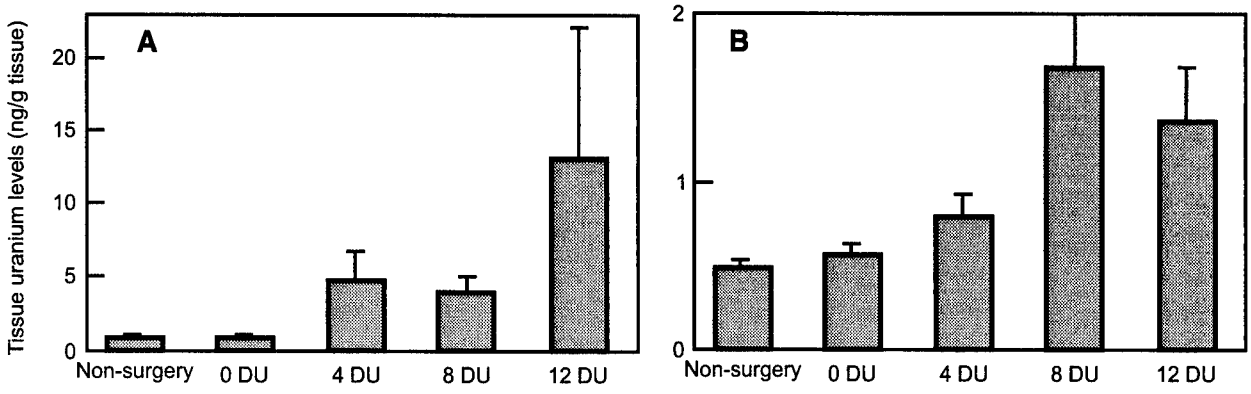

Figure 1. The placental (A) and whole fetus (B) uranium levels.

the DU on maternal parameters such as maternal food and water intake, weight gain during pregnancy, and time-to-pregnancy. Furthermore, the litter parameters such as number of pups, number of males vs. females, and the various levels of DU also did not affect fetal weights. The DU pellets did not adversely affect the ability of these rats to breed, or to maintain the pregnancy until the day of euthanasia. All litters were examined for any overt signs of teratology, and none were noted.

Uranium Distribution. Figure 1A and B show the placental and whole fetus uranium levels. Comparison of these results by a correlation-trend test indicates that uranium accumulates in these tissues in an increasing fashion as the maternal DU dose increases.

Figure 2 shows that a dose-response relationship is also evident in the uranium levels found in the dam's kidneys. However, the kidney levels did not achieve the level we had anticipated as being necessary for reproducing the effects seen by previous researchers—a minimum kidney level of 0.7 μg/g. Our highest DU level averaged approximately 0.5 μg/g U in the maternal kidney.

Figure 3 shows the fetal liver uranium levels. No effect from maternal treatment was seen on uranium levels in the fetal liver tissue.

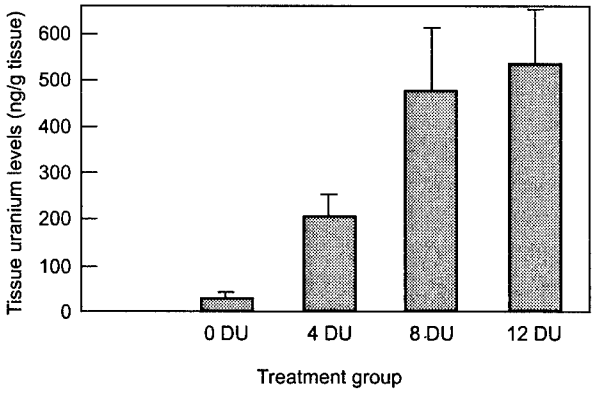

Figure 2. Uranium levels in the maternal kidney.

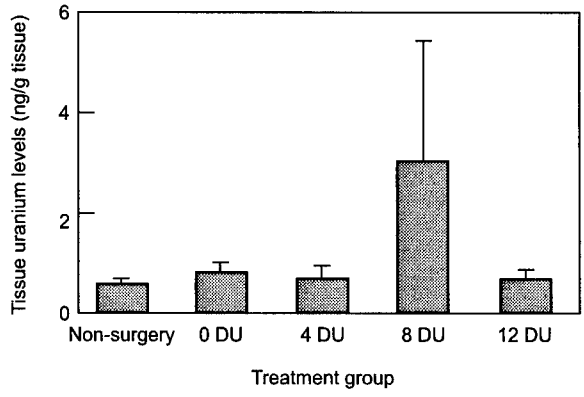

Figure 3. Uranium levels in the fetal liver.

Figure 4 shows the serum uranium levels obtained in the maternal blood. While the measurable levels are low, the correlation-trend test indicated a trend for increasing uranium levels in the blood as the maternal DU dose increased.

Conclusions

The results suggest a dose-response effect on uranium levels in the placenta, whole fetus, maternal kidney and maternal serum. However, the kidney uranium content did not achieve the level we had anticipated as being necessary for reproducing the teratological effects seen by previous researchers—a minimum kidney level of 0.7 μg/g. Our highest DU level only averaged approximately 0.5 μg/g U in the maternal kidney. The uranium did not impact the ability of the rat to breed or to carry the litter to term.

The results of this preliminary study have posed more questions than answers. The DU level in the kidneys of our highest dose did not approach the minimum level known to be nephrotoxic. Future attempts will be made to achieve and possibly exceed this minimum level of 0.7 μg/g. This may be done by increasing the DU dose via increased numbers of implanted pellets, or by allowing the implanted pellets to remain longer before the rats are bred. It is possible that a longer time period is needed for the uranium levels to stabilize. Our attempts to breed the rats soon after surgery may have actually hindered our ability to achieve an equilibrium in our preliminary study. While previous work in our laboratory indicated that urine levels began to stabilize in the 7- to 14-day period, within which we attempted to breed the female rats, we now feel that a 45- to 60-day period is optimal (which would correspond to a 1-year exposure in human females). This time period will allow blood levels to reach a steady state. Since the fetuses were exposed to uranium via the blood, it is vital that blood levels stabilize prior to impregnation.

While these data are preliminary, the fact that uranium was detected in the placenta and whole fetus tissues indicates the potential for developmental toxicity. Fetal exposure to uranium during critical

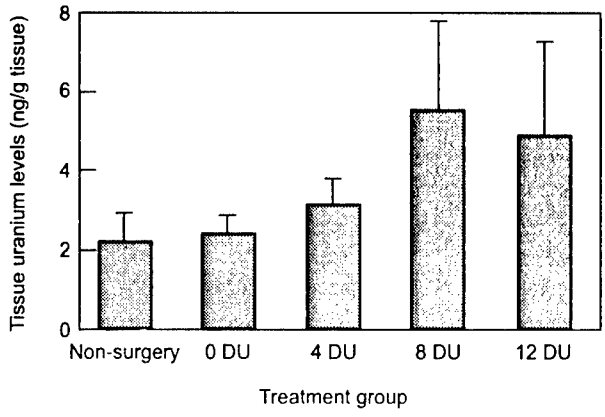

Figure 4. Uranium levels in the maternal serum.

prenatal development may adversely impact the future behavioral and neurological development of offspring. Currently, this laboratory is examining this possibility. We are also investigating the effects that pregnancy has on the toxicology, distribution, and uranium levels in the female rat.

References

1. Bosque MA, Domingo JL, Llobet JM and Corbella J (1993) Embryotoxicity and teratogenicity of uranium in mice following subcutaneous administration of uranyl acetate. Biol. Trace Element Res., 36: 109-118.

2. Domingo JL, Paternain JL, Llobet JM, and Corbella J (1989c) The developmental toxicity of uranium in mice. Toxicology, 55: 143-152.

3. Domingo JL, Ortega A, Paternain LP and Jacinto C (1989b) Evaluation of the perinatal and postnatal effects of uranium in mice upon oral administration. Arch. Environ. Health, 44: 395-398.

4. Paternain JL, Domingo JL, Ortega A, and Llobet JM (1989) The effects of uranium on reproduction, gestation, and postnatal survival in mice. Ecotoxicol. Environ. Safety, 17:291-296.

5. Biological Effects of Ionizing Radiation (BEIR IV) (1988) Health Risks of Radon and Other Internally Deposited Alph-Emitters, 367-395.

6. Durbin PW (1976) Metabolism and Effects of Uranium in Animals, U.S. Energy Research and Development Administration, 68- 129.

7. Waddell WJ and Marlowe C (1981) Biochemical regulation of the accessibility of teratogens to the developing embryo. In: The Biochemical Basis of Teratogenesis, Juchau MR (ed.), Elsevier/North Holland, NY, 1-62.

8. Sikov MR and Mahlum DD (1968) Cross-placental transfer of selected actinides in the rat. Health Phys., 14: 205-208.

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Depleted Uranium Distribution and Carcinogenesis Studies

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Quantitation of the long-term health risk from exposure of humans to uranium (U), particularly in the form of embedded fragments, is complex and involves both chemical and radiological components, as well as possible foreign-body effects. Because of the unique features of the exposures of soldiers in Operation Desert Storm, it is not currently possible to confidently predict the carcinogenic risks to these soldiers from their U-bearing wounds. Such predictions are necessary, however, to guide the medical management of soldiers with U-bearing wounds both now and in the future.

To assess the carcinogenic risks associated with long-term exposure to DU-shrapnel wounds, we are conducting studies in rodents to determine the carcinogenicity of radioactive depleted uranium-titanium alloy [DU(Ti)] fragments in tissues relative to nonradioactive metallic foreign-body fragments. Once a relative carcinogenicity factor is determined in rodent-model systems, it can be used to compare the carcinogenicity of DU(Ti) with the known carcinogenicity of metal fragments in humans. One rodent test system being considered for the carcinogenesis study is the initiation and promotion model of induction of foreign-body tumors in the subcutis of rats or mice. The development of sarcomas near the site of subcutaneous implantation of metal foils, glass slides, or polymer films is well characterized [1]. Several physical characteristics of the implanted materials are important in foreign body carcinogenesis in rodents [1]. Smooth surfaces with a relatively large area appear to be essential for a foreign body to be carcinogenic. Therefore, if the surface of the DU(Ti) foil to be used is altered when in the subcutis of rats and mice, or if the foils are reduced in size through dissolution, the long-term consequences may be changed from those expected.

The purpose of the pilot study reported here was to determine the following: (1) the *in vivo* solubility of DU during the first 60 days after implantation in rats and mice, (2) any changes in the surface characteristics of the DU foil after implantation, and (3) histological responses of rats and mice to the implanted DU during this time. This information is critical for planning relative carcinogenesis studies using these rodents. Two types of foils containing DU were used. One contained only DU, while the other was DU alloyed with 0.75% Ti. The DU foils (20 mm x 15 mm x 1.6 mm) and DU(Ti) foils (20 mm x 15 mm x 1.5 mm) were obtained from Manufacturing Sciences Corporation (Oak Ridge, TN). Tantalum (Ta) foils (Goodfellow Corp., Berwyn, PA) of similar size (22 mm x 15 mm x 1.1 mm) were used as the control implants. The composition of the DU(Ti) pellets is the same as that used in a study at the Armed Forces Radiobiology Research Institute (AFRRI) on the dissolution of DU(Ti) pellets in rats [2] and has been described in detail [3].

The experimental design for the *in vivo* portion of this study is summarized in table 1. Twenty-eight 12-week-old male F344 rats (Charles River Laboratories, Wilmington, MA) and 28 12-week-old male CBA/J mice (Harlan Sprague-Dawley) were used. Animals were housed in filter-topped polycarbonate cages on hardwood chip bedding or in metabolism cages. Animal rooms were maintained at 20 to 22° C with a 40 to 60% relative humidity on a uniform 12-hour light cycle. Food (Lab-Blox, Allied Mills, Chicago, IL) and water were available *ad libitum.*

Table 1. Experimental design for the study of dissolution and excretion of uranium and early biological effects of subcutaneously implanted DU, DU(Ti), or Ta metal.

	Foils in male rats and mice						
	Foil type and number of animals euthanized at 30 d			Foil type and number of animals euthanized at 60 d[a]			
Rodent	DU	DU(Ti)	Ta	DU	DU(Ti)	Ta	Total
F344 rats	5	5	4	5	5	4	28
CBA/J mice[b]	5	5	4	5	5	4	28
Total	10	10	8	10	10	8	56

[a]Twenty-four hour urine samples were collected from three rats and three mice on days -2, -1, 1, 2, 3, 4, 7, 14, 21, 28, 35, 42, 49, 56, and 60 before and after DU and DU(Ti) foils were implanted and on days -2, 7, 14, 28, and 35 before and after Ta foils were implanted.
[b]Deaths of animals prior to the scheduled euthanasia time are discussed in the text.

The metal foils were weighed (mean foil weights ± SD; DU 8.4 ± 0.3 g, DU(Ti) 7.4 ± 0.2 g, and Ta 5.6 ± 0.1 g) and surgically inserted in the subcutis of the upper part of the back region of rats and mice while under halothane anesthesia. A sterile field was prepared over the anterior dorsum; and a surgical incision about 2.5 cm long in the skin was made to place sterile foils into the subcutis. The surgical site was closed with surgical wound clips, which were removed about 7 days after surgery. Twenty-four hour urine samples were collected from rats and mice housed in metabolism cages as indicated in table 1.

Rats and mice were euthanized (table 1) using a sufficient amount of pentobarbital given by intraperitoneal injection, and were necropsied. The tissue capsules around the metal foils were removed, along with the heart-lung block, liver, kidney, femur, urinary bladder, epididymis, testes, and lesions, and were fixed with 10% neutral buffered formalin. Tissues were sectioned at 5 μm; and sections were stained with hematoxylin and eosin. The left kidney, tissue capsule, and lesions were examined histopathologically.

The daily U excretion data for individual rats is summarized in figure 1. The urinary excretion of U appeared to increase throughout the study in rats with implanted DU foils. In contrast, the excretion of U by rats with implanted DU(Ti) increased rapidly

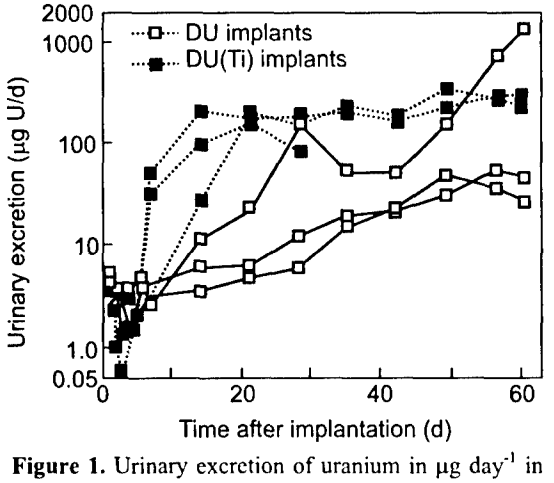

Figure 1. Urinary excretion of uranium in μg day^{-1} in individual rats with DU or DU(Ti) implants.

for 15 to 20 days after which the daily excretion was relatively constant. Similar excretion patterns were seen in mice (data not presented), except that the rate of excretion of U in the mice implanted with DU(Ti) was not constant after 15 to 20 days, as in the rats, but continued to increase. The dissolution of U in the mice with the implanted DU(Ti) led to the accumulation of toxic levels of U in the kidneys and resulted in the death of all but one mouse within 30 days—compared with the death of only one mouse with implanted DU. In contrast, only one rat with a DU(Ti) implant died, but not until day 33. The DU(Ti) foils were more soluble in both mice and rats than were the DU foils in either species. The translocation of U to the kidney and skeleton also indicated that the DU(Ti) foils were more soluble than the DU foils. Histopathological examinations of the kidneys showed a chronic tubular necrosis. The necrosis was severe enough to cause death before the scheduled 60-day euthanasia. The severity of the lesions was generally correlated with the concentration of uranium in the kidney.

Thirty days after implantation in the subcutis, the physical appearances of both the DU and the DU(Ti) foils were markedly altered. The surfaces were roughened and friable, with small black particles or flakes coming off the foils. The flaked particles blackened the lining of the connective tissue capsule surrounding the foils. This appearance was slightly accentuated at the 60-day euthanasia. Histopathological examination of the tissue capsules surrounding the implants showed marked differences between the DU and DU(Ti) foils, and the Ta foils. Around the Ta foils there was a thin connective tissue capsule containing a scant infiltration of chronic inflammatory cells in some of the animals. The DU and DU(Ti) foils were surrounded by a moderately thick connective tissue capsule with moderate infiltration of chronic inflammatory cells. Black particles were found embedded in the capsules. With DU foils, the particles were angular flakes; with DU(Ti) foils, the particles were small and granular.

This work indicates that DU and DU(Ti) foils dissolve more rapidly in mice and rats than was expected; and that DU(Ti) dissolves more rapidly than DU. In addition, both types of DU foils break down in the subcutis, becoming roughened and causing a moderate inflammatory cell infiltration in the surrounding tissues. DU(Ti) foils caused more inflammation and more renal damage. Both of these effects may relate to the greater solubility of DU(Ti). It is evident from these results from both species that the subcutaneous foreign-body carcinogenesis system described by Brand et al. [1] cannot be applied to a study of the carcinogenesis of implanted foils containing DU. Key elements in the Brand system are a smooth surface and a relative lack of inflammation. Therefore, results of the pilot study indicate that the bioassay carcinogenesis study in rats should be conducted using intramuscular implants of DU(Ti) in the form of small pellets and fragments, rather than in the form of foils.

The DU(Ti) pellets and fragments to be used in the carcinogenesis study (table 2) approximate the size of some of the DU(Ti) fragments imbedded in soldiers wounded in the Gulf War. Cylindrical DU(Ti) pellets (2.0 mm long x 1.0 mm in diameter) have been obtained from Manufacturing Sciences Corporation, Oak Ridge, TN. Two sizes of DU(Ti) fragments (2.5 mm x 2.5 mm x 1.5 mm and 5.0 mm x 5.0 mm x 1.5 mm) will be cut from DU(Ti) foils similar to those used in our pilot study.

Fragments (5.0 mm x 5.0 mm x 1.1 mm) of Ta (Goodfellow Corp., Berwyn, PA) will be used as a negative control. Thorotrast® (Hyden Chemical Corp, NY) will be used as a positive carcinogenic control. The distribution, retention, and late effects of ThO_2 used as a radiographic contrast medium in people have been summarized by the New York Academy of Sciences [4] and elsewhere. The experimental design for the 2-year carcinogenesis study is summarized in table 2. A total of 344 12-week-old male Wistar rats (Charles River Laboratories, Wilmington, MA) will be used in this study. The Wistar strain was chosen because, in contrast to the F344, it has a relatively low incidence of nephropathology that could confound the results [5]. The Wistar rat is also larger than the F344, with a larger muscle mass for implantation. In addition, survival and tumor incidence data are available [6]. Fifty rats per group will be required except for the euthanasia series groups (table 2).

The rationale for the 50 rats per group is that this is the standard group size in the National Toxicology Program Statement of Work and the EPA Guidelines 40 CFR 798: 3320— "Combined Toxicity and Oncogenicity Testing." The dose (implant size) has been revised so that this study will be consistent with that used by AFRRI in a 12-month study in rats, i.e., the surface area of the 5 mm x 5 mm x 1.5 mm fragment of DU(Ti) is similar to that which resulted in weight loss in the rats with the implanted fragments.

Before implantation surgery, the DU(Ti) pellets, DU(Ti) fragments, and Ta fragments (table 2) will be weighed, cleaned by immersion in an industrial detergent, rinsed in absolute ethyl alcohol, sterilized by immersion in a 50% nitric acid solution for 3 minutes, rinsed with sterile water, and placed in acetone to inhibit oxidation. This is the same procedure AFRRI employed in their research [2]. These procedures completely remove the oxide formation from the surface of DU metal [7].

Rats will be entered into this study in three blocks of 96 to 102 rats each and one block of 44 rats. Those in the first three blocks will be observed for 2 y, euthanized, and examined histologically. As noted below, urine samples will be collected from a limited number of these rats throughout this study. Forty-four rats having either 2.5 mm x 2.5 mm x 1.5 mm DU(Ti) fragments implanted or having experienced only sham implant surgery will constitute the last block. These rats will be serially euthanized at intervals to 18 months for dosimetry, hematology, clinical chemistry, and histopathology.

Twenty-four hour urine samples will be collected from six rats with four 5.0 mm x 5.0 mm x 1.5 mm DU(Ti) fragments and from six rats with four 5.0 mm x 5.0 mm x 1.5 mm Ta implants. The urine samples will be collected daily for the first 7 days after implantation, twice per week from days 8-28, once per week from days 29-90, and once every 2 weeks from day 91 through 2 years (table 2). Rats with the implanted Ta fragments will serve as controls to

Table 2. Experimental design for a 2-year carcinogenesis study of DU(Ti) pellets and fragments.

	Intramuscularly implanted in male Wistar rats		
Type of implant	Size	Number of implants	Total number of rats[a]
DU(Ti) pellets	2.0 mm x 1.0 mm dia.	4	50
DU(Ti) fragments	2.5 mm x 2.5 mm x 1.5 mm	4	50
DU(Ti) fragments	5.0 mm x 5.0 mm x 1.5 mm	4	50[b]
DU(Ti) fragments	2.5 mm x 2.5 mm x 1.1 mm	4	36[c]
Thorotrast injection	0.025 ml	2	50
Ta fragments	5.0 mm x 5.0 mm x 1.5 mm	4	50
Sham implant surgery	NA	5	8[c]
Sham implant surgery	NA	0	50
Total number of rats			344[d]

[a]Any rats that die within 48 hours of the implantation surgery will be replaced. Sixteen rats will be ordered as spares. Unused spare rats will be euthanized.

[b]Urine samples to be analyzed for uranium will be collected at selected intervals from 6 rats after implantation of the metal fragment from each of these two experimental groups. These rats will not be scheduled for serial euthanasia.

[c]Serial euthanasia of rats, dosimetry, hematology, clinical chemistry, and histopathology: 4 rats at each of the following intervals after implantation of DU(Ti) fragments: 1 week, and 1, 2, 4, 6, and 9 months. Six (6) rats will be euthanized at 12 and 18 months. The 5-implant surgery controls will be euthanized at 18 months.

[d]All surviving rats will be euthanized 2 years after implantation of the metals.

determine the background level of uranium in urine samples. Twenty-four hour urine samples will also be collected from six rats with four implanted DU(Ti) pellets on the same schedule through the 90-day time point (table 2). At that time, all urine samples will be analyzed for uranium. The results will be reviewed and a decision made regarding the need for additional urine samples from the six rats with the DU(Ti) pellets.

In conclusion, we have demonstrated that the subcutaneous implant carcinogenesis system described by Brand et al. [1] is not appropriate to study the carcinogenesis of implanted DU(Ti) fragments in rats. An alternate method, intramuscular implantation of DU(Ti) fragments, will be used to study the carcinogenic effects of implanted DU(Ti) fragments.

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References

1. Brand KG, Buoen LC, Johnson KH, Brand I (1975) Etiological factors, stages, and the role of the foreign body in foreign body tumorigenesis: a review. Cancer Res. 35(2): 279-86.

2. Castro CA, Benson KA, Bogo V et al. (1996) Establishment of an animal model to evaluate the biological effects of intramuscularly embedded depleted uranium fragments. AFRRI Technical Report 96-3. Armed Forces Radiobiology Research Institute, Bethesda, MD.

3. Daxon EG (1995) in: Health consequences of service during the Persian Gulf War: Initial findings and recommendations for immediate action. Washington, D.C., National Academy Press, 59-60.

4. Dahlgren S (1967) Effects of locally deposited colloidal thorium dioxide. Ann. N. Y. Academy Sci. 145:786-790.

5. Gray JE (1986) Chronic progressive nephrosis, rat, in: Monographs on pathology of laboratory animals. TC Jones, U Mohr and RD Hunt (eds) Berlin, Springer-Verlag, 174-179.

6. Bomhard B *et al.* J. Environ. Pathol. Taxicol. Oncol. 7:35-52, 1986; Walsh KM and Porteracki J (1994) Spontaneous neoplasms in control Wistar rats. Fundam. Appl. Toxicol. 22: 65-72.

7. Tonry LL (1993) Solubility of depleted uranium fragments within simulated lung fluid. Doctoral Dissertation, Boston University, Boston, MA

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The Depleted Uranium Follow-Up Program Baltimore VA Medical Center

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The first large-scale use of uranium-containing munitions occurred in the Persian Gulf War (PGW). Depleted uranium (DU) had for some time been incorporated into both projectiles and armor by the U.S. military and other countries who valued its density, availability, and relative cost. It has reduced radioactivity, and as a by-product of the uranium enrichment process, is readily abundant. Many military personnel were around uranium munitions during and immediately after the PGW. Individuals who were on or in vehicles at the time the vehicles were struck by uranium projectiles were the most significantly exposed. A clinical assessment of those exposed personnel has demonstrated that while there are generally few adverse health effects detected other than the shrapnel and burn injuries, some of the survivors have ongoing uranium absorption from retained shrapnel in addition to their acute inhalation exposure. A second round of clinical evaluation is planned for these soldiers; and the content of the evaluation will be expanded. The uptake and distribution of uranium is in some ways analogous to other heavy metals such as lead, mercury, arsenic, and cadmium. Less is known, however, about the toxicology of uranium. Research has focused on the nephrotoxicity it shares with the other heavy metals. [1].

There are few studies on the reproductive and developmental toxicity of uranium. Uranium has been shown to cause both developmental [2,3] and reproductive abnormalities, including embryo lethality in mice [4,5]. The majority of animal studies show no histological damage to the gonads. However, testicular damage in rats has been associated with large amounts of uranyl nitrate in the diet [6,7]. Uranium in the form of uranyl nitrate (UO^2_{+2}) has been documented to be genotoxic in *in vitro* studies of Chinese hamster ovary (CHO) cells—causing increases of micronuclei, sister chromatid exchange (SCE), and chromosomal aberrations (CA) [8].

The reproductive effects of uranium in humans have been poorly studied. Uranium is known to cross the placental barrier in animals; therefore, exposure to high concentrations of uranium may expose the developing fetus [1]. In exposed human populations, chromosomal aberrations in a cohort of uranium miners have been documented [9]. A more recent study of uranium-production workers documented significant increases in both SCE and CA, which the authors attributed to the chemical nature rather than to the compound's radiologic hazard [10].

The impact of mutagenic exposure on reproductive function is well documented [11,12]. Some studies of uranium miners show an alteration in the sex ratio of live males to live females being born; but these findings are difficult to interpret since several effect modifiers (e.g., socioeconomic class, race, and medical histories of the miners and their spouses) were not considered [13–15].

In 1992 a medical surveillance follow-up study was initiated at the Baltimore VA Medical Center to further examine a group of 33 PGW veterans who were struck by or otherwise exposed (through inhalation or ingestion) to armor-piercing anti-tank shells composed of depleted uranium. Results of that evaluation did not reveal any clinically significant health abnormalities related to uranium exposure other than the sequelae of their traumatic injuries; but a number of veterans were found to have elevated urinary concentrations of uranium.

A second round of follow-up is planned for the spring of 1997. At that time a clinical assessment of reproductive function of the DU-exposed cohort will be performed. Collection of information on medical, occupational, and reproductive history obtained by questionnaire from both the DU-exposed cohort and their spouses or partners will be undertaken. With informed consent, we will obtain sperm and serum samples from men enrolled in this study as well as from two sets of appropriate controls. Parameters to be evaluated include physical characteristics as well as concentration and morphology of the sperm. Functional characteristics including mobility, speed of forward progression, and evaluation of sperm survival will be determined.

Serum hormone levels (testosterone, FSH, LH, prolactin) will be measured using radioimmunologic techniques. Peripheral blood lymphocytes will be used to examine sister chromatid exchanges; chromosomal aberrations will be used to examine any potential mutagenic effect from DU exposures. Serum hormone levels and semen parameters will be evaluated as a function of urinary uranium concentration and whole-body uranium counting results. These uranium measures will serve as independent variables against which the chromosomal, hormonal, and semen parameters (independent variables) will be assessed. Questionnaire data will assist in identifying possible confounders and in documenting personal risk factors related to reproductive function.

References

1. Toxocologic Profile for Uranium (1990) Agency for Toxic Substances and Disease Registry, U.S. Public Health Service.

2. Domingo JL, Paternain JL, Llobet JM *et al.* (1989a) The developmental toxicity of uranium in mice. Arch Environ Health 44:395-398.

3. Domingo JL, Ortega A, Paternain JL *et al.* (1989b) Evaluation of the perinatal and postnatal effects of uranium in mice upon oral administration. Arch Environ Health 44:395-398.

4. Paternain JL, Domingo JL, Ortega A *et al.* (1989) The effects of uranium on reproduction, gestation and postnatal survival in mice. Exotox Environ Saf 127:291-296.

5. Llobet JM, Sirven J, Ortega A, Domingo JL (1991) Influence of chronic exposure to uranium on male reproduction in mice. Fundam Appl Toxicol 16(4):821-829.

6. Malenchenko AF, Barkun NA, Genseva GF (1978) Effect of uranium on the induction and course of experimental autoimmune orchitis and thyroiditis. J. Hyg Epidemiol, Microbiol, Immunol 22:268-277.

7. Maynard EA, Downs WL, Hodge HC (1953) Oral toxicity of uranium compounds. In: Voegtlin, Hodge HC, Eds. Pharmacology and toxicology of uranium compounds. New York, NY: McGraw-Hill.

8. Lin R, Wu L, Lee C, Lin-Shiaw S (1993) Cytogenetic toxicity of uranyl nitrate in Chinese hamster ovary cells. Mutat Res. 319(3):197-203.

9. Brandom WF, Saccomomanno G, Archer VE *et al.* (1978) Chromosome aberrations as a biological dose-response indicator of radiation exposure in uranium miners. Radiat Res 76:159-171.

10. Martin F, Earl R, Tawn EJ (1991) A cytogenic study of men occupationally exposed to uranium. Brit J Ind Med 48:98-102.

11. Kucerova M, Gregor V, Horacek J, Dokinska M, Matejckova S (1992) Influence of different occupations with possible mutagenic effects on reproduction and level of induced chromosomal aberrations in peripheral blood. Mutat Res. 278:19-22.

12. Russell L, Aaron C, de Serres F, Generoso W, Kannan K, Shelby M, Springer J and Voytek P (1984) Report of the U.S. Environmental Protection Agency Gene-Tox program. Evaluation of mutagenicity assays for purposes of genetic risk assessment. Mutat Res. 134:143-157.

13. Muller C, Ruzicka-Jaroslav Bakstein L (1967) The sex ratio in the offspring of uranium miners. Acta Universitatis Carolinse Medica 13(7/8):599-603.

14. Waxweiler RJ, Roscoe RJ, Archer VE (1981) Secondary sex ratio of firstborn offspring of U.S. uranium miners. In: Weise W, Ed. Birth defects in the Four Corners area. Transcripts of Meeting, February 27, 1981, Albuquerque, NM, 37-50.

15. Weise WH, Skipper BJ (1986) Survey of reproductive outcomes in uranium and potash mine workers: Results of first analysis. Ann Am Conf Gov Ind Hyg 14:187-192.

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In Vivo X-Ray Fluorescence (XRF) Measurement of Depleted Uranium

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This study investigates the applicability of x-ray fluorescence (XRF) in measuring bone uranium concentrations in soldiers exposed to this heavy metal during the Gulf War. The system designed uses a ^{57}Co source to excite the uranium x-rays—with the source and detector in an approximate 180° backscatter geometry relative to the sample position. It is demonstrated (by experiment and Monte Carlo simulation) that the x-ray to coherent-peak ratio is linearly related to concentration and is independent of variations in source-sample geometry, thickness of overlying tissue, and tibia size. Preliminary *in vivo* measurements in volunteers from the general population indicate a minimum detectable concentration (MDC) of approximately 20-ppm, which may not be sufficiently sensitive for measuring this cohort. The first measurements of this group indicate that XRF can identify localized uranium fragments beneath the skin but failed to measure bone uranium levels above the detection limit.

Introduction

The toxicological effects of uranium have long been appreciated. Individuals at risk have been monitored for uranium exposure for many years. However, the complex metabolic pathway from uranium inhalation/ingestion to sites of retention makes accurate body-burden assessment a difficult task. Typically, individuals are monitored with lung scans and urine samples; but this is generally not considered an effective method of assessing long-term exposures.

As with many heavy metals, uranium accumulates mainly in bones and kidneys with prolonged exposure. Therefore, an *in vivo* method of measuring uranium in these sites should provide a more accurate index for cumulative and target-organ exposure than can be obtained from urine samples and lung scans. Such a measurement technique has been developed for the detection of *in vivo* heavy metal concentrations such as lead in bone [1], cadmium [2], mercury [3], gold [4], and platinum [5] in kidneys using XRF. This study analyzes the potential use of XRF in measuring metabolized uranium stored in bone and the role of XRF in identifying the presence of subcutaneous uranium-containing fragments.

Experimental Procedure

Part I: Measuring Metabolized Uranium Stored in Bone. The system employs a 1 mCi ^{57}Co source to excite the uranium x-rays in an approximate backscatter geometry (figure 1). This results in the Compton

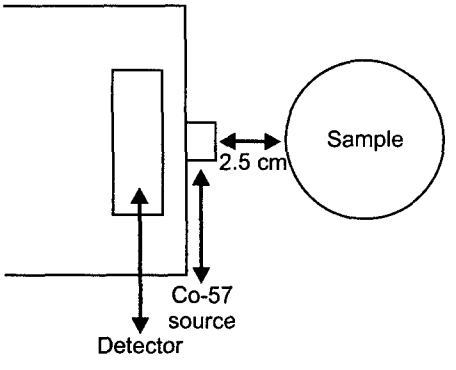

Figure 1. Experimental setup in backscatter geometry using a 1 mCi ^{57}Co source to excite the uranium x-rays.

peaks being located at lower energies than the uranium x-rays—with acceptable separation for background minimization. The detector is of hyperpure germanium, 50.5 mm in diameter and 20 mm thick with a resolution of 700 eV at 122 keV. The detector output is passed through the usual fast nuclear electronics with the final output displayed as a digitized spectrum on a computer monitor (figure 2).

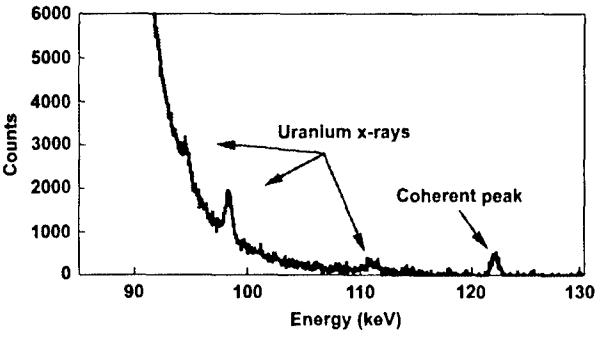

Figure 2. The final output is displayed as a digitized spectrum on a computer monitor. The spectrum shown here was generated from a 100 ppm uranium plaster of Paris phantom.

Six uranium doped plaster of Paris phantoms were prepared with concentrations from 0 to 100 ppm. Each phantom was irradiated three times in random orientations to assess uniformity. Each spectrum was divided into four regions, alpha (1 and 2), beta 1 and 3, beta 2′ and 2″, and the coherent region upon which a non-linear least squares Marquardt method [6] was used to analyze the intensities of three x-ray peaks and the coherent peak. The ratios of the $K\alpha_1$, $K\beta_1$, and $K\beta_2$ x-ray intensities to the coherent intensity were plotted against uranium concentration (figure 3). (Note: The legitimacy of normalizing to the coherent peak to eliminate geometric factors in the relationship between x-ray intensity and uranium present was established with a Monte Carlo simulation. A more detailed description of this work is available [7].

A similar procedure was used in measuring 10 volunteers, males and females, 22 to 49 years old, 9 with no occupational exposure and 1 with 10 years experience working with uranium in a university setting. Each measurement required an acquisition time of 45 minutes live time, which corresponds to approximately 50 minutes with 10% dead time. Mean concentrations were calculated from the x-ray to coherent ratios using the established calibration lines, making allowance for differences between plaster of Paris and bone mineral. The bone uranium concentration of one member of the Gulf-War cohort has been assessed for both left and right tibia and right calcaneus sites. These measurements required 45 minutes live time each; and the mean concentrations were calculated from the x-ray to coherent ratios as before.

Part II: Identifying Uranium Fragments. This portion of the work involved the use of a specially designed phantom to simulate the deposition of uranium fragments in various geometries. The phantom was constructed from tissue-equivalent plastic cast into six circular sections, each approximately 40 mm thick, having a diameter of 150 mm. The sections are fastened by a bone phantom, cylindrical in shape, that can be thread through the tissue sections at the center or at a position that is 55 mm offset from the center. This is to allow for the simulation of limbs with either central or offset bore location, i.e., a femur or a tibia. Each section has a specific distribution of drilled channels into which tissue-equivalent plastic plugs can be placed. These plugs were used to position the uranium pellets manufactured for this project, ranging in mass from 8 to 60 mg. The phantom was then irradiated with the system described in section I. The spectra were analyzed to determine the intensity of x-rays detected in a 1-hour period.

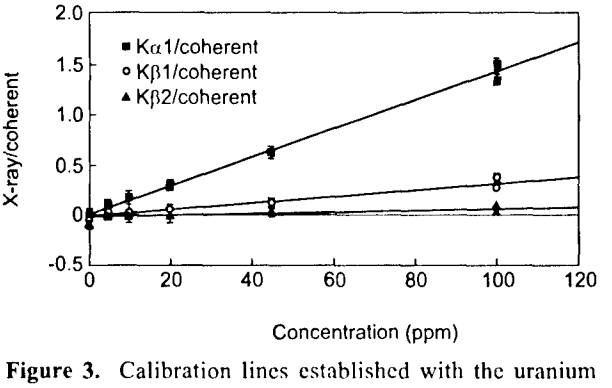

Figure 3. Calibration lines established with the uranium plaster of Paris phantoms. The slope and intercept of each line can then be used to determine the mean uranium concentration from the measured x-ray to coherent ratios *in vivo*.

One member of the cohort was also measured to identify the presence of uranium-containing fragments. Unfortunately we did not have access to the x-ray films of this individual during the XRF examination and had to rely on the subject's memory of the location of fragments for starting positions. Three such locations were examined to qualitatively determine the presence or absence of subcutaneous uranium.

Results and Discussion

Part I: Measuring Metabolized Uranium Stored in Bone. From the *in vivo* measurements of the 10 volunteers, the average concentration was 4.0 ppm with a standard deviation of ±13.1 ppm. With a two-tailed t test this was assessed to be not significantly different from a population mean of 0 ppm at the 5% significance level. The average uncertainty for individual results was approximately 9 ppm. This is a useful measure of precision and suggests that the *in vivo* MDC is on the order of 20 ppm for this system. Studies of uranium levels in bone measured from autopsy samples from both occupational and non-occupational groups [8–10] found that non-occupational groups (from 20 studies worldwide) range in concentrations from 0.8 to 20 ng/g_{ash}, with a mean value of roughly 8 ng/g_{ash}; whereas the occupationally exposed subjects (sample size of 8 workers) ranged in concentration from 0.05 to 1.8 $\mu g/g_{ash}$ (note: 1 ppm is equivalent to 1 $\mu g/g_{ash}$).

Based on this small sample of uranium workers, one can conclude that the typical concentration in both non-exposed subjects and uranium workers is beyond the capabilities of this system. While this particular cohort does appear to have a continuous supply of uranium in the transfer compartment (urine levels are fairly constant over a period of a few years), the urine levels are not significantly higher than levels seen in occupationally exposed subjects. This implies that these individuals can not be expected to have significantly higher bone-uranium concentrations than occupational levels. Therefore, without further improvements to the system, it is not expected that XRF will be useful in the clinical examination of bone-uranium concentrations in this cohort.

The measurements on the first subject from the group confirm this expectation. The bone uranium was assessed in the right tibia and calcaneus (right leg known to not contain fragments based on radiographs) and two measurements were performed on the left tibia. Three of these four measurements resulted in concentrations that were not above the detection limit. The fourth measurement, one of the assessments of the left tibia, resulted in an equivalent concentration of 86 ± 14-ppm. This will be discussed in the following subsection. Preliminary dose estimates for this system indicate the subject receives an effective dose equivalent of roughly 30 nSv in a 1-hour measurement.

Part II: Identifying Uranium Fragments. Figure 4 illustrates the results of the measurement of x-ray intensity for two pellets, 32.5 and 62.5 mg, respectively, positioned from 5 to 55 mm beneath the surface of the phantom. It is apparent there is a rapid decrease in signal intensity as the overlying tissue thickness increases. It is also apparent that the fragments are observable with as much as 3 to 4 cm of overlying tissue masking the signal. Therefore, XRF is a feasible method of qualitatively assessing whether fragments contain uranium, provided the shrapnel is within 3 to 4 cm from the surface with a measurement time on the order of 1 hour.

However, figure 4 also illustrates an important observation. The x-ray intensity observed does not appear

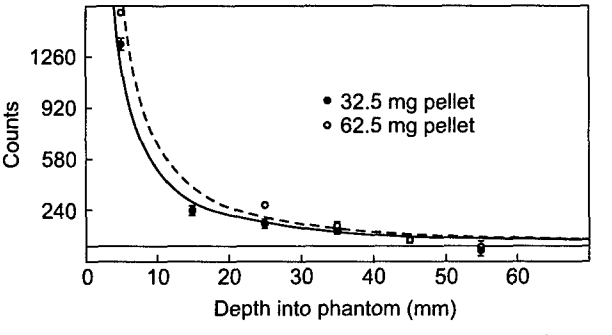

Figure 4. X-ray intensity from uranium fragments as a function of depth in the phantom. Note that the large difference in uranium mass does not result in a correspondingly large difference in the x-ray intensity at any depth. It is suggested that this is a result of self-attenuation. This will make calibration extremely difficult if the surface area to mass ratio is the important parameter as the shapes of these fragments are unknown in our cohort.

to have a strong relationship to the mass of the fragment. It is believed that this is due to self-attenuation. The larger fragment does not result in a proportionally larger number of x-rays observed because the increase in size associated with this increase in mass results in more x-rays being attenuated before leaving the surface of the sphere. It has been suggested that the number of x-rays detected is more likely to depend on the actual mass per unit surface area of the fragment. We hope to investigate the x-ray intensity dependence on fragment shape in the near future. However, because there is no clear relationship between x-ray intensity and fragment mass, there is no clear method of calibration to allow XRF to quantitatively assess the amount of uranium identified in the subcutaneous fragment. Future work will involve assessing the extent to which calibration can be achieved in determining an effective mass per unit surface area based on x-ray intensity and an estimate of fragment depth.

As mentioned above, one of the four bone-uranium measurements resulted in a non-zero equivalent bone concentration. We suggest that this is due to signal arising from a uranium-containing fragment within the tissue region above the tibia site that was being measured. This is the likely source of the signal since the other three measurements of bone concentration were not significantly different from zero, including a measurement of the same bone a few centimeters below the site in question. Also there was some scar tissue on the surface of the skin at this location; and the left leg was known to contain shrapnel from the Gulf-War injuries. The other three attempts at identifying uranium fragments in this subject did not indicate any presence of this metal; however, we were limited by time and the choice of location within the left leg based on the subject's memory.

Conclusions

Initial measurements with a member of this cohort serve to summarize the current capabilities of the XRF system. Seven measurements were made in total—three attempting to identify uranium in subcutaneous fragments and four attempting to assess the amount of metabolized uranium stored in the bone. Of these seven measurements, only one resulted in a measurable quantity of uranium being detected—measured during one of the four bone uranium assessments. The spectrum was analyzed as previously discussed and was determined to be equivalent to 86 ± 14 ppm. Since the three other bone sites resulted in concentrations that were not significantly different from 0 ppm, it was concluded that this non-zero measurement was in fact the localization of a uranium-containing fragment. This first measurement of a member of the cohort illustrates that XRF is capable of qualitatively identifying the presence or absence of uranium in subcutaneous fragments. However, it does not appear to be sufficiently sensitive at this point for assessing the metabolized bone-uranium concentrations accumulating from injuries from the Gulf War.

References

1. Somervaille LJ, Chettle DR, Scott MC (1985) *In vivo* measurement of lead in bone using x-ray fluorescence. Phys Med Biol 30(9):929-43.

2. Christoffersson JO and Mattsson S (1983) Polarised X-rays in XRF-analysis for improved *in vivo* detectability of cadmium in man. Phys Med Biol 28(10):1135-44.

3. Börjesson B, Barregård L, Sällsten G *et al.* (1995) *In vivo* XRF analysis of mercury: the relation between concentrations in the kidney and the urine. Phys Med Biol 40(3):413-26.

4. Shakeshaft J and Lillicrap S (1993) Technical note: an X-ray fluorescence system for the determination of gold *in vivo* following chrysotherapy. Br J Radiol 66(788):714-7.

5. Jonson R, Mattsson S, Unsgaard B (1988) A method for *in vivo* analysis of platinum after chemotherapy with cisplatin. Phys Med Biol 33(7):847-57.

6. Bevington PR (1969) Data Reduction and Error Analysis for the Physical Sciences. New York: McGraw Hill.

7. O'Meara JM, Chettle DR, McNeill FE, Webber CE (1997) The feasibility of measuring bone uranium concentrations *in vivo* using source-excited x-ray fluorescence. Phys Med Biol 42:1109-1120.

8. Wrenn ME, Durbin PW, Howard B *et al.* (1985) Metabolism of ingested U and Ra. Health Phys 48(5):601-633.

9. Leggett RW (1994) Basis for the ICRP's age-specific biokinetic model for uranium. Health Phys 67(6):589-610.

10. Dang HS, Pullat VR, Sharma RC (1995) Distribution of uranium in human organs of an urban Indian population and its relationship with clearance half-lives. Health Phys 68(3):328-31.

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Feasibility Studies of a Method for Determining Depleted Uranium Deposited in Human Limbs

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Whole-body counters have been used for many years to monitor exposure to uranium. The γ-ray signals specific to uranium-decay chains are counted; and their signal strength is then related to exposure by comparing them to calibration standards. However, γ-ray intensities depend not only on the mass of uranium present, but also on its location. The technique used to measure uranium content only provides a quantitative estimate when calibration standards are constructed that model both the uranium level and the distribution. Thus, to quantify the mass of depleted uranium, such as shrapnel, embedded in a limb is problematic, as neither the uranium level nor the distribution is known. Even if the size and location of large pieces of shrapnel can be inferred from radiographs, it is still unclear whether the pieces are actually DU or a combination of DU and other metals. Also, some fragments are too small to be discerned by x-ray imaging. Therefore, although the presence of DU can be detected, quantitative estimates require further information.

It was suggested that the technique of differential attenuation could be used to provide the extra information about DU location required for an accurate estimate of DU mass. This technique relies on the fact that different γ-ray energies are attenuated differently by varying thicknesses of overlying tissue. The determination of the ratio of γ-ray signals at different energies would thus provide distribution information and, in combination with the γ-ray signal strengths, could be used to calculate DU masses. A study was performed to determine whether the technique could locate and quantify depleted uranium pellets within a phantom. Measurements were performed in a whole-body counter using hyperpure germanium detectors. These detectors are required for differential attenuation studies due to their good energy resolution. Depleted uranium pellets ranging in size from 8.8 mg to 62.5 mg were placed in various locations inside a phantom that simulated a limb. The phantom was made of Rando soft tissue-equivalent material surrounding a central bone-equivalent shaft. It was designed to allow for the placement of pellets at multiple locations within the material. The differential attenuation measurements were based on the 93 keV γ-ray from ^{234}Th and the 1 MeV γ-ray from ^{234m}Pa; these energies are far enough apart to have very different attenuation characteristics in tissue.

This technique could detect DU pellets in the phantom. A minimum detectable limit of 0.50 ± 0.02 mg was calculated from studies of the 8.8-mg pellet. However, inconsistencies were discovered in signals from the same pellet measured at the same location, depending on the pellet orientation. These may have been due to the pellets being newly manufactured and not having achieved secular equilibrium at the surfaces. In addition, self-attenuation of the pellets was found to be a problem. Finally, the precision of the 1 MeV γ-ray measurements was relatively poor because of the reduced efficiency of germanium detectors at this energy.

In conclusion, the system could be used to identify depleted uranium pellets within a phantom down to very low levels. The differential attenuation measurements did provide limited information as to size and location. However, further work is required to determine if the technique will be feasible for

human subjects. Future studies will focus on checking the secular equilibrium of the pellets, the use of various detector positions to locate shrapnel, and techniques to improve counting efficiency.

Round-Table Discussion

Major Conclusions

- In the Department of Vetrans Affairs patient-monitoring program, no functional changes in the patients have been observed to date; therefore, there is no indication that a change in the clinical approach to care and treatment for these previously injured patients is warranted.

- It was agreed that a more aggressive approach for the removal of DU fragments is necessary. If DU fragments are suspected and are removable, they should be taken out within the first 6 months following the initial injury. This change from previous policy is based on laboratory animal evidence of heavy-metal toxicity.

- To expedite removal, it was suggested that medical doctrine be changed to include a probe in the fielded medical kits to identify these types of injuries (DU) quickly so that fragments can be removed as soon as possible.

- The use of chelators to reduce the DU burden was discussed. The consensus was that chelators would not currently be used to treat a patient with embedded solid-metal fragments. The danger of mobilizing the metal and increasing the distribution is too high. In addition, current chelators can themselves cause kidney damage.

- No evidence yet exists for kidney damage in animals chronically exposed to DU; although the kidney uranium levels are three times the levels known to cause damage in acutely exposed rats.

- The time- and dose-dependence of oncogene expression in animal models is a cause for concern. Further animal studies on carcinogenesis and close observation of the current DVA patients is warranted.

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Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

During Operation Desert Storm (ODS) friendly-fire incidents resulted in patients wounded from embedded fragments of depleted uranium (DU) metal. Existing fragment removal guidelines dictated fragments be left in place unless they were a present or future threat to health. An Armed Forces Radiobiology Research Institute (AFRRI) 1993 review of the potential health effects of allowing DU fragments to remain in place found no compelling evidence to warrant a change in the fragment removal policies. However, sufficient uncertainties existed concerning the health effects of embedded DU fragments to warrant implementation of both patient follow-up and toxicological research programs. The Department of Veterans Affairs (DVA) is conducting a joint DoD/DVA patient monitoring effort; and the DoD is funding a DU research program at AFRRI and at the Inhalation Toxicology Research Institute (ITRI). A meeting of these groups was held at AFRRI 15 November 1996 to review research efforts to date. This report is a summary of the eight research efforts presented at the workshop.

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